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Phytotoxicity of Lead on the Physiological Parameters of Two Varieties of Broad Bean (*Vicia faba*)

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***Abstract*—**The phytotoxicity of heavy metals can be expressed on roots and visible part of plants and is characterized by molecular and metabolic answers at various levels of organization of the whole plant. The present study was undertaken on two varieties of broad bean *Vicia faba* (Sidi Aïch and Super Aguadulce). The device was mounted on a substrate prepared by mixing sand, soil and compost, the substrate was artificially contaminated with three doses of lead nitrate [Pb(NO3)2] 0, 500 and 1000 ppm. Our objective is to follow the behavior of plant opposite the stress by evaluating the physiological parameters. The results reveal a reduction in the parameters of the productivity (chlorophyll and proteins production) with an increase in the osmoregulators (soluble sugars and proline).These results show that the production of broad bean is strongly modified by the disturbance of its internal physiology under lead exposure.

***Keywords*—**Broad bean, lead, stress, physiological parameters, phytotoxicity.

I. Introduction

ONE of the main issues in environment is the lead contamination of the atmosphere, water and the soils. This metal cannot be biodegrade and persists in the environment for long periods. To concede like physiologically nonuseful [1], lead is potentially toxic for the living organisms, and it can induce a degradation of the biological activity [2], the plants and water quality, even with weak concentration. It leads to the inhibition of germination and the root elongation, also; it leads to the reduction in the production of biomass and the photosynthetic activity, inducing a delay of growth, a drop of production and with damage on the external morphological level [3]-[6]. The various tests performed in the present work, focuses on the estimation of the effects of lead on the physiological behavior of two varieties of broad bean at various developmental stages.

II. Materials and Method

A. Preparation of the Substrate

The substrate is prepared based on a mixture of sand, soil and compost in proportion of (8-3-1).The soil has been taken from the farm of the university of Tiaret on a piece in fallow for several years, it was taken from a 30 cm depth in order to avoid any possible atmospheric lead contamination, the ground dried with the free air is filtered by a sieve of 2 mm. The substrate is put in cylinders out of PVC of 900 mm height and 110 mm diameter. Each cylinder is closed in its basal side by fabric filter of 1 mm diameter of the pores. A sample of the soil is analyzed to determine these physicochemical properties (Table I). These analyzes were carried out at the regional laboratory of the National Institute of the Soils and the Irrigation and Drainage of Ksar Chellala-Tiaret, Algeria.

TABLE I
Physicochemical Property of the Soil Used

Granulometry (Pipette Of Robinson):	
< 2µm	05.26%
2 µm to 20 µm	07.89%
20 µm to 50 µm	44.18%
50 µm to 200 µm	17.52%
200 µm to 2 mm	25.15%
pH (AFNOR X31-103 Soil/Water:2/5)	07.67
Electric conductivity (Iso:11265 Soil/Water:1/10)	0.416 (Ms/Cm)
Organic matter (Walkley Method)	0.13%
Nitrogen Total (Kjeldahl ISO:11261)	0.18%
Total limestone (Calcimeter Bernard ISO:10693)	04.41%
Active limestone	0.75%
Cation exchange capacity (CEC)	10 Meq/100g of Soil
U.S.D.A Texture:	Silt Loam

B. Vegetable Materiel

The seeds of Vicia faba of the two varieties Sidi Aïch (V1) and Super Aguadulce (V2) are carefully provided by the Technical Institute of the Field Crops of Sidi-Bel-Abbes, Algeria. The seeds are rinsed with water then plunged during fifteen minutes in a sodium hypochlorite solution with 6% to eliminate any possible fungal contamination. After several rinsings with water to eliminate the remainders from sodium hypochlorites, the seeds were put to germinate in food plasticboxes, on compacted absorbing paper, soaked with water and raised by polystyrene plates of 1.5 cm. After that, the boxes were placed in a cupboard, at darkness, in room temperature and with a high humidity for three to four days.

C. Crop Management

The young plants after four days of germination were planted on a device made up of three levels, with ten repetitions for each level for the two varieties. The device was placed under semi-controlled greenhouse. The irrigation is currently carried out every two days in order to ensure the maintaining of the ground with the field capacity. The nutritive solution is brought once all the three irrigations. The plants are preserved under greenhouse until the end of the experimentation for 60 days duration (Fig. 1).

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Fig. 1 Experimental device (on the right Sidi Aïch variety, and on the left Super Aguadulce variety)

D. Addition of Lead

Only one application was carried out, 25th day after the seeds transplanting. This application comprises two amounts (level of treatment) 500 ppm (D.2), 1000 ppm (D.3) and with a control amount (D.1).Lead is brought in the form of lead nitrates Pb(NO3)2.

E. Measured Parameters

1. Proportioning of the Chlorophyll Pigments

The concentrations of chlorophyll are determined by spectrometry according to the procedure quoted by Lichtenthaler [7].

The following equations are used to calculate the concentrations of chlorophyll in the sheets (in mg/g) [8]:

- chlorophyll a = 12,25 × A663 - 2,79 × A645 (chloro A)
- chlorophyll b = 21,50 × A645 - 5,10 × A663 (chloro B)
- chlorophyll a+b = 7,50 × A663 + 18,71 × A645(chloro A+B)

2. Proportioning of Soluble Sugars

Simple sugars (glucose, fructose, and saccharose) are extracted by a solvent able to solubilize them and block the enzymatic activities apt to degrade them. They are proportioned by the method of [9].

3. Total Protein Rate of Seeds

The total protein determination is carried out after determination of total nitrogen by the method of [10] according to the following formula:

Total proteins (%) = NR % X 6.25 [11].

4. Proportioning of the Proline

The followed method is that of [12], simplified and developed at the point by [13].

F. Statistical Analyzes

The results are presented in averages ± standard deviation and studied by stat box version 6.40 in order to arise variance with two factors in randomization, the effect being significant when $p<0.05$.

III. Results

A. Content of Chlorophyll Pigments

For the values of the rate of chlorophyll has (Table III) one announces a reduction according to the amount of lead for the V2 starting from D3, with 6.86 (mg/g) for the control, 6.81 (mg/g) for D2 and 3.97 (mg/g) for D3.In the same way for V1, the reduction in the values of chlorophyll is recorded only starting from D3 (7.65 mg/g) and a slight increase 10.30 (mg/g) for D.2 against 9.72 (mg/g) for the control. Moreover, one notices that the values recorded for V1 are more important than those recorded for V2.The variance analysis (Table II) watch an effect very highly significant of variety factor on the rate of chlorophyll has ($p < 0.001$) in the same way for lead dose factor. For the rates of chlorophyll B (Table III) the results show a progressive reduction for V2 according to the amount of lead, 1.53 (mg/g) for D.1, 1.13 (mg/g) for D.2 and 0.83 (mg/g) D.3.For V1 one records a slight increase in chlorophyll B for D.2 1.98 (mg/g) against 1.75 (mg/g) for the control and 1.36 (mg/g) for D.3.The V1 variety records values higher than those of V2. The chlorophyll A+B rates follow in a great part the rates of the chlorophyll A, these values (Table III) present for V1 an increase for D.2 of 12.28 (mg/g) against 11.47 (mg/g) for D.1, then they fall down to 9.01 (mg/g) for D.3. For V2 the values record a reduction according to the amount which is very important for D.3 of order 4.80 (mg/g) against 8.39 (mg/g) for D.1 and 7.94 (mg/g) for D.2.It is also announced that V2 records inferior rates with those recorded for V1.The variance analysis shows an effect very highly significant ($p < 0.005$) of variety factor and for the lead dose factor.

TABLE II
VARIANCE OF THE PHYSIOLOGICAL PARAMETERS

Variables	Pb dose (F1)	Variety (F2)	Interaction (F1xF2)
Chloro A	0*	0.001*	0.837ns
Chloro B	0.403ns	0.754ns	0.917ns
Chloro A+B	0*	0*	0.463ns
Total proteins	0*	0*	0*
Proline	0.031*	0.016*	0.628ns
Soluble sugars	0.631ns	0.014*	0.472ns

ns: insignificant *: significant.

TABLE III
CHLOROPHYLL RATE DEPENDING ON THE DOSE OF PB FOR V1 AND V2

Variety	Pb dose (ppm)	ChloroA (mg/g)	Chloro B (mg/g)	ChloroA+B (mg/g)
V1	0	9.72 ±3.59	1.75 ± 2.68	11.47 ± 1.93
	500	10.30 ±1.66	1.98 ± 2.27	12.28 ± 1.84
	1000	7.65 ±1.81	1.36 ± 3.27	9.01 ± 2.64
V2	0	6.86 ±2.79	1.53 ± 2.58	8.39 ± 1.28
	500	6.81 ±0.92	1.13 ± 1.69	7.94 ± 1.28
	1000	3.97 ±1.91	0.83 ±1.64	4.80 ± 1.08

B. Soluble Rates Sugars

For the contents of soluble sugars the results show an increase according to the amount for the two varieties, namely 72.86 (µg/g) against 83.62 (µg/g) for D.1, 117.81 (µg/g) against 99.62 (µg/g) for D.2 and finally, 131.71 (µg/g) against 163.14 (µg/g) for D.3.The variations according to the variety are not visible owing to the fact that the values remain brought closer (Fig. 2). V2 records the greatest value for the D.3 amount with 163.14 (µg/g) of average.

The variance analysis shows a significant effect of lead dose factor on the sugar content with a probability $p < 0.05$ (Table III).

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Open Science Index, Agricultural and Biosystems Engineering Vol:9, No:8, 2015 publications.waset.org/10002206.pdf

C. Rate of Total Proteins

The values recorded for the rate of total proteins register a reduction for V1, they pass from 33.06% for the witness to 24.33% for D.2, and arrive for D.3 at 22.02%, and this reduction also affects the values recorded for V2 which records 36.51% average for D.1, then 33.67% for D.2 and 24.37% for D.3 (Fig. 3). It is also noticed that V2 records rough protein values higher than that recorded for V1.The variance analysis shows that variety factor has an effect very highly significant ($p < 0.005$), lead dose factor with an effect very highly significant ($p < 0.005$), and also an effect very highly significant ($p < 0.005$) for the interaction between the two factors (F1xF2).

D.Content of Proline

The results of proportioning of the proline show an increase according lead dose for the two varieties with 220.49 (D.3), 103.70 (D.2) and 100.12 (µg/g) for D.1 (for V1) and, 123.83 (D.3), 62.72 (D.2) and 53.95 (µg/g) for D.1 (for V2) (Fig. 4). The variance analysis shows a significant effect of variety factor with a threshold of probability $p < 0.05$, in the same way for lead dose factor with a significant effect proportions ($p<0.05$).

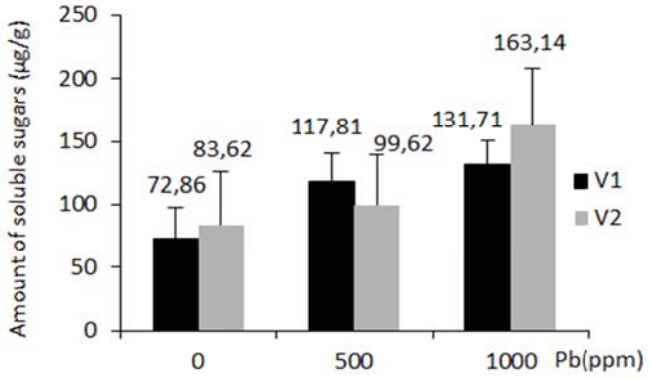

Fig. 2 Evolution of Soluble Sugars Amount According Lead Dose for V1 and V2

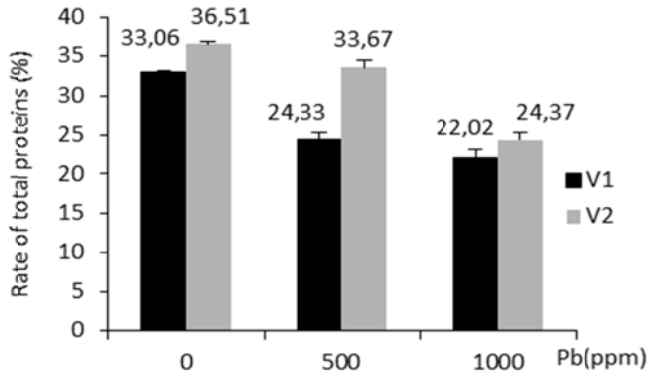

Fig. 3 Evolution of Total Proteins Rate According Lead Dose for V1 and V2

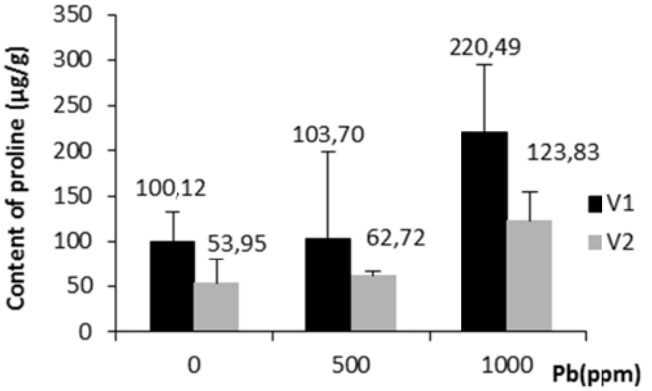

Fig. 4 The Proline Evolution According Lead Dose for V1 and V2

IV. Discussion

The results obtained show a significant regression on the rates of the chlorophyll according to the lead amount present in the medium, the work of [14] shows that lead can decrease the rate of chlorophyll, but this reduction remains weak contrary to the reduction observed in the presence of Zn and Cu. In the same way, the results also showed a reduction in the rates of pigments; this reduction seems to be very significant for chlorophyll A and chlorophyll A+B (the reduction in chlorophyll A+B is the result of the reduction in chlorophyll A). Chlorophyll A seems more sensitive to Pb action, than chlorophyll B these results are in agreement with the results obtained by [15] and [16]. The reduction of the rates of the pigments (in particular chlorophyll A and chlorophyll A+B) is In agreement with the work completed on the broad bean [4], [8], [17]. The results obtained in this study and which show also that the application of lead is accompanied by a clear accumulation of soluble sugars. This accumulation remains however conditioned by the amount of lead and the time of its application. The results of this study showed a reduction in the rate of rough proteins according to the added lead amount (effect very highly significant). Super Aguadulce variety records protein values largely higher than those recorded by Sidi Aïch due probably to a varietal criterion.

The results obtained in this study, show a relative increase in the contents of proline according to the increase in the lead amounts applied in the substrate. The accumulation of the proline was recorded in the presence of strong amounts of lead in accordance with [17]-19], applied to plants of broad bean, or other species [14], [20].

V. Conclusions

The present study revealed notable disturbances on the plan physiological of broad bean. On the one hand, this study showed a reduction in biosynthesis parameters (photosynthetic activity and rate of total proteins) according to the intensity of lead applied in the substrate. In the other hand, the results recorded in this study revealed an increase in the osmoregulators (proline and soluble sugars) which are accumulated following the presence of lead in the substrate. The presence of lead in the medium induced severe behavioral

modifications on the physiological level of broad bean.

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